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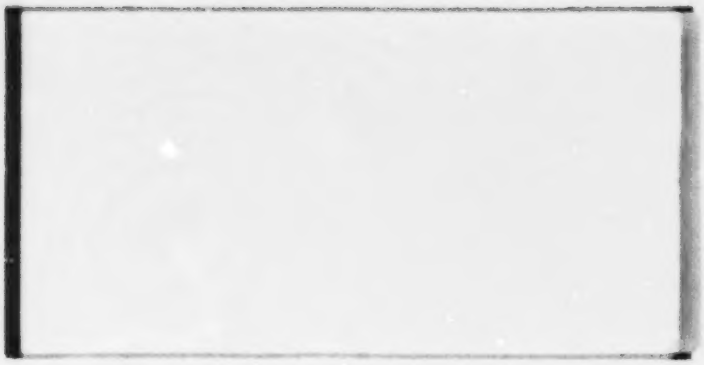

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# ABSORPTION, TRANSLOCATION AND EFFECT ON SURVIVAL OF 2,4-D, PICLORAM AND TRICLOPYR IN WHITE ASH AND CAROLINA POPLAR AFTER SOIL-APPLICATION

Report No 85-264-K

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## ABSTRACT

Although the uptake and translocation of herbicides has been studied in woody plants there have been few attempts to relate the concentration of herbicide within the root system, the ultimate target in any herbicide application program, and the effectiveness of that herbicide in the long-term. In the present study, application of the herbicides 2,4-D plus picloram and triclopyr to the soil in which Carolina poplar and white ash trees were growing resulted in the differential inhibition of several leaf and root metabolic processes in the short-term, and death of the trees of both species in the long-term. The white ash trees, which received proportionally more herbicide than the Carolina poplar because the former are more herbicide-resistant, had proportionally higher concentrations of the herbicides in their roots. Respiration in root segments from white ash was less sensitive to inhibition by the herbicides than the respiration of Carolina poplar.

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EXECUTIVE SUMMARY

# ABSORPTION, TRANSLOCATION AND EFFECT ON SURVIVAL OF 2,4-D, PICLORAM AND TRICLOPYR IN WHITE ASH AND CAROLINA POPLAR AFTER SOIL-APPLICATION

G.P. Arron
Biological Research Section
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Ontario Hydro uses various herbicides as part of a selective control program to control the growth of brush and woody species on rights-of-way. The most commonly used herbicides are 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DP (2,4-dichlorophenoxy propionic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid). The concentrations of herbicide within the root that are necessary to cause death of the complete root system in but few of these species have been determined.

In the present study, the uptake of 2,4-D, picloram and triclopyr [(3,4,5-trichloro-2-pyridinyl)-oxy] acetic acid by roots of white ash *(Fraxinus americana L.)*, a herbicide-resistant species, and Carolina poplar *(Populus X canadensis* Moench), a herbicide-sensitive species, was correlated with short-term effects on leaf and root metabolism, and the long-term survival of the trees in the field. The herbicides were applied to the soil in which the trees were growing.

The mid-summer application of the herbicides 2,4-D plus picloram or triclopyr to the soil in which two-year old Carolina poplar and white ash trees were growing in the greenhouse resulted in the death of all the trees, as indicated by the lack of leaf production in the following spring. The death rate among untreated trees was extremely low.

The concentration of herbicide found in the root tissue of both species was related to the amount of herbicide applied to the soil. White ash trees, which received proportionally more herbicide than Carolina poplar trees, had proportionally higher

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concentrations of herbicides in their roots when measurements were made 3 and 10 days after herbicide application. In both species, the concentration of picloram in the roots was significantly higher after 10 days than after 3 days, while such a time-dependent uptake was less apparent with triclopyr and least apparent with 2,4-D. The concentrations of 2,4-D, picloram and triclopyr found in the leaves of white ash were greater than those found in Carolina poplar leaves, and this may merely reflect the fact that more herbicide was found in the roots of the former.

From the results obtained in this study there is no evidence to suggest that the herbicide-resistance of white ash is related to a non-entry of herbicide into the roots. A differential sensitivity of leaf and root metabolism to inhibition by the herbicides was apparent. Transpiration was inhibited in both species after only 2 days (while herbicide concentrations in the leaves were relatively low), while root segment respiration appeared less sensitive to the herbicides. Carolina poplar root respiration was inhibited by all herbicide treatments after 10 days but not 3, while with the exception of the high application rate of triclopyr, no inhibition of respiration in white ash was observed. Given the high concentration of herbicides in the white ash roots, this lack of inhibition may reflect the fundamental high degree of tolerance of herbicides that this species exhibits. In the long-term however, the concentrations of 2,4-D plus picloram and of triclopyr found in the roots of both white ash and Carolina poplar were effective in causing the death of the trees, but the minimum concentrations necessary to cause death cannot be defined.

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To Mr. F.J. Kee
Director of Research

# ABSORPTION, TRANSLOCATION AND EFFECT ON SURVIVAL OF 2,4-D PICLORAM AND TRICLOPYR IN WHITE ASH AND CAROLINA POPLAR AFTER SOIL-APPLICATION

## 1.0 INTRODUCTION

Ontario Hydro uses various herbicides as part of a selective control program to curb the growth of brush and woody species on rights-of-way. The most commonly used herbicides are 2,4-D (2,4 -dichlorophenoxyacetic acid), 2,4-DP(2,4 -dichlorophen-oxypropionic acid) and picloram (4-amino-3,5,6-trichloro-picolinic acid). Combinations of these herbicides (eg 2,4-D plus 2,4-DP or picloram plus 2,4-D) are applied as a foliar treatment during the spring and summer months. Picloram is also applied in pellet form by aircraft as a soil application (for root pickup) in several isolated areas.

Certain woody species encountered on Ontario Hydro rights-of-way, such as white ash *(Fraxinus americana L)*, are more resistant to these herbicides than are others. In the case of foliar-applied herbicides, it has been suggested that resistance may be linked to a lack of penetration of herbicide through the cuticle and into the leaf cells. Davis et al (1968a) reported that only 2% of the picloram applied to leaves of live oak *(Quercus virginiana* Mill) was absorbed after 48 hours. Herbicide resistance may be linked to a lack of movement of herbicide out of the treated leaf, even after penetration of the leaf cells. Davis et al (1968b) found that 99% of the picloram taken up by leaves of velvet mesquite *(Prosopis juliflora)* remained in the leaf cells 90 hours after application.

Of the herbicide applied to the foliage of a plant, only small quantities appear to be translocated to the roots. Mitchell and Stephenson (1973) reported that of the $^{14}C$-picloram applied to a mature leaf of either white ash, a herbicide-resistant species, or red maple (*Acer rubrum* L), a herbicide-sensitive species, 97% in both cases remained in or on the leaf, and 1.18% and 0.81% respectively, reached the roots of the tree after 12 days. Thus it appeared that herbicide tolerance was not related to the non-arrival of herbicide in the roots. Since the concentration of picloram was higher in the white ash roots, these authors concluded that the roots of different species differ in their sensitivity to picloram. In more recent studies (OHRD Report No 83-178-K) in which 2,4-D plus 2,4-DP, picloram plus 2,4-D, and triclopyr [(3,4,5-trichloro-2-pyridinyl)-oxy] acetic acid were applied to the foliage (to leaf drip) of white ash and Carolina poplar (*Populus* X *canadensis* Moench), a herbicide-sensitive species, very little herbicide was detected in the roots of the trees. Low levels of 2,4-D (when applied with 2,4-DP) and triclopyr (2.3 ± 0.5 and 3.0 ± 0.5 $\mu$g per g root fresh weight respectively) were detected in the Carolina poplar roots, but no picloram or 2,4-DP. Picloram (1.1 $\mu$g per g root fresh weight) was detected in the roots of one white ash tree (of three examined), but 2,4-D,2,4-DP and triclopyr were not detected in any samples. In a similar experiment conducted with the same tree species, in which $^{14}C$ labelled 2,4-D and 2,4-DP were applied to the foliage, the distribution of label in the foliage and roots, up to 40 days after application suggested that the resistance of white ash (to 2,4-D and 2,4-DP) was attributable to poor translocation of the herbicide to the roots. Only one component of the mixture applied, 2,4-D, was found in the roots of Carolina poplar (OHRD Report No 83-537-K). In both species most of the $^{14}C$-labelled material was recovered from the leaves (and leaflets in the case of white ash) that had either been treated or were adjacent to treated leaves.

In considering the experiments described above in which herbicide uptake and translocation were studied after foliar application of herbicide, it is apparent that there are two significant problems with such studies. In most instances the amount of herbicide applied is difficult to determine in that spraying to 'leaf drip' means that the amount of herbicide sprayed on is always greater than that retained. Secondly, the amount of herbicide reaching the roots or alternatively the ultimate concentration of the herbicide in the roots, has been correlated with the effectiveness of the herbicide in killing those roots in but a few species. From the work of Mitchell and Stephenson (1973) it is apparent that the roots of different species do differ in their sensitivity to the herbicide picloram. The question then arises as to what is the concentration of the particular herbicide or combination of herbicides in the roots

of the various woody species found on Ontario right-of-way that result in root and tree death? Given this knowledge it would then be a matter of ensuring that enough herbicide gets into the roots of the various species (by whatever means) to exceed these known thresholds.

The present study attempts to relate the concentration of the herbicides 2,4-D and picloram (applied as a mixture in Tordon 101) and triclopyr (applied alone) found in the roots of Carolina poplar and white ash after soil-application of the herbicides, to short-term effects on leaf and root metabolism and to the survival of those trees over the long term.

## 2.0 MATERIALS AND METHODS

### 2.1 Herbicide Application

Two-year old white ash (*Fraxinus americana* L) and Carolina poplar (*Populus X canadensis* Moench) trees were a gift from the Ontario Ministry of Natural Resources (collected from the Midhurst Station). The former were approximately 0.5 m in height and the latter approximately 2 m. The white ash trees were planted in 30 cm pots in a one and one half to one mixture of shredded topsoil (native sandy loam) and peat moss (Plant Products, Bramalea, Ontario) and the Carolina poplar trees were planted in 37 cm pots in the same mixture. The trees were placed in the greenhouse, watered twice a week and fertilizer (solution of NPK - obtained from Plant Products) applied every two weeks. Approximately three to four weeks after planting 95% of the trees of both species had broken dormancy. Herbicides were applied to the trees (see below) five to six weeks after breakage of dormancy.

The herbicides Tordon 101 (a mixture of picloram and 2,4-D) and triclopyr were obtained from the Dow Chemical Company, Sarnia, Ontario. A detailed description (lot number, concentration etc) of these herbicides has been given previously (Ontario Hydro Research Division Report No 83-178-K).

Each stock herbicide solution was diluted with tap water to give four treatment solutions. Two treatment solutions, containing either a high or a low concentration of the herbicides were used with white ash and two with Carolina poplar. The solutions used with white ash contained more herbicide than the equivalent solutions (i.e. high or low concentration) used with Carolina poplar as the former species is more resistant to herbicides.

The amounts of herbicide in each treatment solution were chosen after a preliminary experiment had been conducted in which 1,10,100 or 1000 mg active ingredient of either Tordon 101 or triclopyr were applied to individual pots containing either a white ash or a Carolina poplar tree. This approach was similar to that used by Mitchell and Stephenson (1973) who exposed the roots of red maple and white ash to a range of concentrations of picloram. From observations of the foliar appearance and measurements of the stomatal diffusion resistance (see below) over the course of about 2 weeks it was possible to determine which herbicide treatments were having a deleterious effect on the leaf metabolism of the two tree species. While the high doses of both Tordon 101 and triclopyr had a harmful effect on Carolina poplar metabolism they had little if any effect on white ash metabolism. As a result of this preliminary experiment it was decided to apply to each Carolina poplar tree 100 mL of solution containing either 0.4 g (active ingredient) 2,4-D plus 0.1 g picloram, 0.04 g 2,4-D plus 0.01 g picloram, 0.48 g triclopyr or 0.048 g triclopyr. To each white ash tree was applied 100 mL of a solution containing either 2.4 g 2,4-D plus 0.6 g picloram, 0.24 g 2,4-D plus 0.06 g picloram, 2.88 g triclopyr or 0.288 g triclopyr. For both white ash and Carolina poplar the respective lower application rates of 2,4-D plus picloram or triclopyr were applied to 27 trees, and the higher rates to 21 trees. For each species 21 trees formed the control group and in total 117 trees of each species were used.

Prior to application of the respective herbicide solutions, each tree was watered liberally to ensure that the herbicide moved down through the complete soil column and did not become localized in the surface layer only. After herbicide application, watering would result in some herbicide being washed completely through the soil column and out of the pot. Studies by Bovey et al (1979) with picloram and triclopyr indicated that 15% or less of the applied herbicide was lost from the pot by 10 days after herbicide application. After herbicide application in the present study the trees were maintained in one compartment of the greenhouse whilst untreated control trees were maintained in another compartment.

## 2.2 Observations on Herbicide Effects

At regular intervals, notes were made on the appearance of the treated and control trees. Discolouration and loss of turgor of leaves and stems were recorded using a four stage subjective scoring system (0 - no effect, 3-maximum effect). A score of 3 for leaf discolouration, meant that approximately 95% (or more) of the surface of 95% of the leaves were discoloured.

Four weeks after herbicide application the 15 trees that remained from each treatment set after the destructive sampling program described below were taken to the herbicide study area at Wesleyville GS and planted. The survival of these trees was assessed in the summer of the following year when all those which had put out new leaves were judged to be alive.

## 2.3 Determination of Stomatal Diffusion Resistance and Root Respiration

Stomatal diffusion resistance was measured on the lower surface of leaves using an autoporometer (LI-65 Lambda Instruments Corp, Lincoln, Nebraska). At each time interval, the stomatal diffusion resistance was measured in six leaves chosen at random for each treatment of the two species. The autoporometer was calibrated as described in the equipment manual (Lambda Instruments A-176) and a calibration curve used to correct the experimental data to 25°C.

Oxygen consumption by root segments was measured at 30°C in a Rank oxygen electrode system (Rank Bros, Bottisham, Cambridge, UK). Samples of washed tree roots were cut into short sections and placed in 2 mL of 100 mM potassium phosphate buffer (pH 7.0). Oxygen consumption was measured for approximately 5 minutes. After assay the root segments were removed, surface dried and weighed. Oxygen consumption was expressed as nmoles $O_2$ taken up per minute per g fresh weight.

## 2.4 Collection of Plant Tissue Samples, Herbicide Extraction and Analysis

At various times after application of herbicide to the trees, samples of foliage and the complete root system were collected from each of three trees in each treatment set. Root samples were collected by removing the tree from the pot, carefully brushing away the soil and then washing the roots in a bucket of water. After being allowed to drain, the roots were placed in a plastic bag, weighed, and stored at -16°C prior to herbicide extraction. Foliage samples, of ten to fifteen leaves (leaflets in case of white ash) collected at random from each tree, were placed in plastic bags and weighed before storage at -16°C.

Thawed leaf and root tissue was freeze-dried and the latter ground in a Wiley Mill. Between 5 and 15 g of material was extracted with 150 mL of acidified acetone as described by Bovey et al (1979). The resulting ether fractions were submitted to Analytical Services for herbicide analysis.

The residues of 2,4-D, picloram and triclopyr present in the samples were estimated quantitatively by GLC (gas-liquid chromatography). The herbicide content of each sample was expressed as a function of the dry weight of the plant material extracted.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Herbicide Content Of The Roots Of White Ash And Carolina Poplar Trees After Soil Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr

The herbicide contents of root samples collected from white ash and Carolina poplar trees three and ten days after soil application of the herbicides 2,4-D plus picloram or triclopyr are presented in Tables 1 and 2. The results are expressed on the basis of µg of herbicide per g dry weight of root. Expressing the results on the basis of µg of herbicide per tree showed similar trends to those presented (data not shown).

Several trends are obvious from the data. The concentration of herbicide present in the root samples varies as a function of the amount of herbicide applied to the soil. Thus the more herbicide that is applied the greater concentration of herbicide is found in the roots. This appears to hold both for white ash and for Carolina poplar separately, and when the two species are compared.

The effect that time has on the concentration of herbicide found in the white ash and Carolina poplar roots apparently varies as a function of the herbicide in question. For picloram there appears to be a consistent trend in that for both species of tree and both respective application rates, the concentration of picloram in the roots is significantly higher after ten days than after three days (Tables 1 and 2). Such an increase of herbicide concentration with time is rather less apparent with triclopyr (where in three out of four cases there was a significant increase with time) and not apparent with 2,4-D where there was only one case of a significant increase in concentration with time (Table 1). The apparent increase in picloram concentration in the roots seen with white ash and Carolina poplar has been observed before by Mitchell and Stephenson (1973) who exposed the roots of both white ash and red maple to a nutrient solution containing picloram, and by Bovey et al (1979) who also observed an increase in triclopyr concentration when picloram or triclopyr were applied to the soil surrounding the roots of huisache. Although the latter authors observed a decrease in the concentrations of both picloram and triclopyr from 10 to 30 days after treatment, the former authors observed a further increase in picloram concentration from 12 to 24 days.

TABLE 1

HERBICIDE CONTENT OF ROOTS OF WHITE ASH TREES COLLECTED 3 OR 10 DAYS AFTER SOIL APPLICATION OF VARIOUS AMOUNTS OF EITHER A MIXTURE OF 2,4-D AND PICLORAM OR TRICLOPYR

| Treatment† | Herbicide Detected | Days after herbicide treatment | |
|---|---|---|---|
| | | +3 | +10 |
| | | µg/g dry weight(a) | |
| Control | 2,4-D | - | 0.2 ± 0.2 |
| | Picloram | - | 0.1 ± 0.1 |
| | Triclopyr | - | 0.2 ± 0.1 |
| ALDP | 2,4-D | 5.6 ± 4.1 | 4.2 ± 2.4 |
| | Picloram | 0.5 ± 0.3 | 2.8 ± 1.1+ |
| AHDP | 2,4-D | 30.3 ± 20.7 | 387 ± 77+ |
| | Picloram | 3.5 ± 2.8 | 61.8 ± 24.7+ |
| ALT | Triclopyr | 8.4 ± 0.6 | 11.4 ± 2.3 |
| AHT | Triclopyr | 33.8 ± 28.7 | 248 ± 105+ |

Key: - No samples collected

a Values given are the mean ± one standard deviation (n = 3)

† Abbreviations for treatments are as follows: The low and high rates of 2,4-D plus picloram applied to white ash were 240 mg 2,4-D plus 60 mg picloram (ALDP) and 2400 mg 2,4-D plus 600 mg picloram (AHDP) and to Carolina poplar were 40 mg, 2,4-D plus 10 mg picloram (PLDP) and 400 mg, 2,4-D plus 100 mg picloram (PHDP) respectively. Similarly for triclopyr, white ash received 288 mg (ALT) or 2880 mg (AHT) triclopyr and Carolina poplar 48 mg (PLT) or 480 mg (PHT). In abbreviations: A = white ash; P = Carolina poplar; L = low; H = high; DP = 2,4-D plus picloram; and T = triclopyr.

+ Values within a row followed by this notation are significantly different from the mean value quoted directly to their left, at the p = 0.05 level in a two-tailed t-test.

TABLE 2

HERBICIDE CONTENT OF ROOTS OF CAROLINA POPLAR TREES COLLECTED 3 OR 10 DAYS AFTER SOIL APPLICATION OF VARIOUS AMOUNTS OF EITHER A MIXTURE OF 2,4-D AND PICLORAM OR TRICLOPYR

| Treatment† | Herbicide Detected | Days after herbicide treatment | |
|---|---|---|---|
| | | +3 | +10 |
| | | µg/g dry weight(a) | |
| Control | 2,4-D | - | nd |
| | Picloram | - | 0.3 ± 0.0 |
| | Triclopyr | - | 0.2 ± 0.1 |
| PLDP | 2,4-D | 0.4 ± 0.3 | 1.0 ± 0.4 |
| | Picloram | 0.4 ± 0.1 | 1.2 ± 0.3+ |
| PHDP | 2,4-D | 13.4 ± 7.6 | 13.8 ± 3.9 |
| | Picloram | 3.0 ± 1.5 | 7.6 ± 1.0+ |
| PLT | Triclopyr | 0.4 ± 0.1 | 2.3 ± 0.9+ |
| PHT | Triclopyr | 5.7 ± 2.7 | 40.6 ± 9.1+ |

Key: nd Not detected

- No samples collected

a Values given are the mean ± one standard deviation (n = 3)

† Abbreviations for treatments are described in Table 1.

+ Values within a row followed by this notation are significantly different from the mean value quoted directly to their left, at the p = 0.05 level in a two-tailed t-test.

Changes in the concentrations of 2,4-D, picloram or triclopyr in the roots of the white ash and Carolina poplar may be the result of a number of reactions. Given that there is a large amount of herbicide outside the root that is free to enter as a function of time, then the herbicide concentration might increase with time. However herbicide which has already entered the root is reactive and may have a variety of fates, among them translocation out of the root to the stem and leaves. Alternatively herbicide molecules may be degraded, ring hydroxylated or conjugated with cellular constituents (OHRD Report No 82-153-K) and thus become both metabolically unavailable and not identified in the assay system used in these experiments.

The apparent increase in concentration of picloram and lack of an increase in concentration of 2,4-D in the roots of the two species observed over the first ten days of the present study may be related to differences in the persistence of the two herbicides in soil. Picloram is a persistent herbicide with a half-life of approximately one year (McCall and Agin, 1985) while 2,4-D has a half-life of 4 and 6 days in Ontario soils (Thompson et al, 1984). Thus the amount of 2,4-D available to the roots for uptake may have dropped considerably even over the course of ten days. Conditions in the pots were most conducive to herbicide degradation with high moisture levels, a high organic matter content and high temperatures (Hance, 1980). Triclopyr has a half-life of from 10 to 46 days, and is therefore intermediate between picloram and 2,4-D (Dow, 1984).

The results presented in Tables 1 and 2 also provide information on the relative uptake of 2,4-D and picloram from a mixture applied. Although the ratio of 2,4-D to picloram in the Tordon 101 applied was 4:1 (w/w), such a ratio was only consistently maintained when higher application rates of 2,4-D plus picloram were supplied. In Carolina poplar for example, with a low application rate of 2,4-D plus picloram, there were no apparent differences in the concentrations of the two herbicides at 3 or 10 days, but with the higher application rate differences in concentration were apparent. Such differences may be explained by a more efficient uptake of picloram rather than 2,4-D at low external concentrations, and such a difference may become obscured when external concentrations are higher.

Given that the concentrations of 2,4-D plus picloram and triclopyr detected in the roots of the white ash and Carolina poplar trees that received the low application rates of the herbicides were sufficient to result in death of these trees (see section 3.6 below), how do such concentrations compare with those from previous studies? Bovey and Mayeux (1980) applied the manufacturers' recommended rate of picloram or triclopyr to the soil in which honey mesquite plants were growing and found

concentrations of picloram in the roots of between 2.0 to 3.0 µg fresh weight and of triclopyr of between 0.8 to 1.2 µg fresh weight when measurements were made 3, 10 and 30 days after application. Simulated field applications of 2,4-D plus picloram and triclopyr to foliage of white ash and Carolina poplar resulted in concentrations of 3.0 µg triclopyr per g fresh weight being found in Carolina poplar roots and of 1.1 µg picloram per g fresh weight being found in white ash roots (OHRD Report no 83-178-K). Assuming that dry weight constitutes between 10 and 20% of fresh weight, then such results should be multiplied by either 10 or 5 so they can be compared with those from the present study. The herbicide concentrations from this earlier work (Bovey and Mayeux, 1980; OHRD Report No 83-178-K) are therefore somewhat higher than those given in Tables 1 and 2. These latter values were indeed effective in causing the death of the treated trees in the long term (discussed in more detail below) and since higher concentrations of picloram and triclopyr were found in the roots of white ash and Carolina poplar respectively after foliar-application (OHRD Report No 83-178-K), this would suggest that foliar-application is as effective as soil-application in killing the root system, at least for specific herbicides and species. Perhaps the definitive experiment would be one in which, after foliar- or soil-application, known concentrations of herbicides in the roots are correlated with their long term effects on the root system in the field.

The concentrations of picloram and triclopyr reported by Bovey and Mayeux (1980) after soil application to honey mesquite plants were also effective in causing stem death by twelve weeks after treatment, with triclopyr killing 70% of the stem tissue and picloram 100%. Data on root death were not collected by these authors. Bovey and Mayeux (1980) applied the same amount of either picloram or triclopyr to the soil containing their honey mesquite plants, and thus comparison of the subsequent concentrations of the two herbicides detected in the roots was simple. In the present study, picloram was supplied in combination with 2,4-D, and the amounts of triclopyr and picloram supplied were not equivalent, making direct comparison of the results difficult.

Attempts were made to allow a direct comparison of the results from the present study for the various herbicides and tree species by expressing either the concentration of each herbicide detected in the roots of the trees or the total amount of each herbicide in the complete root system as a function of the amount of that herbicide applied to the soil. No significant differences between the herbicides was apparent when the "efficiency" of root uptake of the various herbicides at 3 or at 10 days was compared in this fashion (data not shown). There was a consistent difference between picloram and triclopyr as against

2,4-D in that while the latter showed no significant increase in either concentration or total amount of herbicide per tree expressed as a function of the total amount of herbicide applied to the soil when 3 day-old samples were compared with 10 day-old, both picloram and triclopyr did.

### 3.2 Herbicide Content Of The Leaves Of White Ash And Carolina Poplar Trees After Soil Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr

Herbicide molecules may be translocated to the stems and leaves of plants after appearing in the root cells. The herbicide contents of leaves of white ash and Carolina poplar trees collected 3 or 10 days after soil application of either a mixture of 2,4-D and picloram or triclopyr are presented in Table 3. The concentrations of the three herbicides are greater in the white ash foliage than Carolina poplar and this may merely reflect the fact that more herbicide was applied to the former than the latter. In both species the concentration of picloram in the leaves was greater after 10 days than after 3, but only when the respective low application rates of picloram were used. Such a time-dependent increase in concentration of picloram was observed previously in the roots of both species for both respective application rates of the herbicide (Tables 1 and 2). The concentration of triclopyr in the foliage of both species also increased between 3 and 10 days but only for the respective high application rates.

Expressing the concentration of each herbicide in the foliage as a function of the amount of that herbicide applied to the soil would give some indication of the efficiency of herbicide movement from the tree roots upwards into the leaves. Analysis of such data revealed that the only significant difference between the herbicides was the efficient transfer of picloram from the soil to the foliage of Carolina poplar where a low rate was applied (data not shown). Mitchell and Stephenson (1973) reported that picloram accumulated in the foliage of white ash to a far greater extent than in the foliage of red maple when the roots of both species were exposed to the same concentrations of picloram. The larger concentration of picloram in the foliage of the herbicide-tolerant white ash was attributed both to a more efficient uptake of the herbicide by the roots and a more efficient translocation of that herbicide from the root to the foliage. Bovey et al, (1979) also reported an efficient translocation of picloram from the roots to the foliage of honey mesquite and huisache, while translocation of triclopyr was much less efficient. Although triclopyr accumulated in the roots of huisache to far higher levels than did picloram, it did not arrive in the leaves to any great extent (Bovey et al, 1979).

TABLE 3

HERBICIDE CONTENT OF LEAVES OF WHITE ASH AND CAROLINA POPLAR TREES COLLECTED 3 OR 10 DAYS AFTER SOIL APPLICATION OF VARIOUS AMOUNTS OF EITHER A MIXTURE OF 2,4-D AND PICLORAM OR TRICLOPYR

| | | Ash Leaves ——Days | | Poplar Leaves after herbicide treatment—— | | | |
|---|---|---|---|---|---|---|---|
| Treatment¶ | Herbicide Detected | +3 -µg/g dry | +10 weight(a)- | Treatment¶ | Herbicide Detected | +3 -µg/g dry | +10 weight(a)- |
| Control | 2,4-D | - | 0.1* | Control | 2,4-D | - | 0.1* |
| | Picloram | - | 0.2* | | Picloram | - | 0.1 ± 0.0† |
| | Triclopyr | - | 0.1 ± 0.1† | | Triclopyr | - | 0.4 ± 0.1 |
| ALDP | 2,4-D | 0.4 ± 0.1 | 1.2 ± 0.2+ | PLDP | 2,4-D | 0.3 ± 0.1 | 0.3 ± 0.1 |
| | Picloram | 0.2 ± 0.0 | 0.4 ± 0.1+ | | Picloram | 0.3 ± 0.1 | 0.7 ± 0.1+ |
| AHDP | 2,4-D | 8.7 ± 5.8 | 12.3 ± 16.0 | PHDP | 2,4-D | 4.3 ± 1.1 | 4.5 ± 0.8 |
| | Picloram | 1.3 ± 1.1 | 2.4 ± 1.5 | | Picloram | 1.2 ± 0.3 | 1.3 ± 0.5 |
| ALT | Triclopyr | 1.2 ± 0.5 | 2.4 ± 1.5 | | Triclopyr | 0.1 ± 0.0 | 0.3 ± 0.4 |
| AHT | Triclopyr | 4.8 ± 2.4 | 11.2 ± 1.2+ | | Triclopyr | 0.1 ± 0.0 | 1.2 ± 0.6+ |

Key:
- * Only one sample (of three) contained herbicide above the detection limit
- † Only two samples contained herbicide above the detection limit
- a Values given are the mean ± on standard deviation (n=3)
- - no samples collected
- ¶ Abbreviations used for treatments are described in Table 1.
- + Values with a row followed by this notation are significantly different from the mean value quoted directly to their left, at the p = 0.05 level in a two-tailed t-test.

It is of interest that while picloram movement up the stem and into the foliage is apparently much more efficient than triclopyr movement, rates of movement of these herbicides down the stem after foliar-application are similar. In both huisache and honey mesquite application of the same rate of picloram and triclopyr to the foliage resulted in similar concentrations of the two herbicides being found in the roots of the plants when samples were collected 3,10 and 30 days after treatment (Bovey et al, 1979, Bovey and Mayeux, 1980).

### 3.3 Effects Of Soil-Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr On The Appearance Of White Ash And Carolina Poplar Trees

The effects of soil-application of the herbicides 2,4-D plus picloram and triclopyr on the appearance of the foliage of white ash and Carolina poplar trees are summarized in Table 4. In the white ash the loss of leaf turgor and leaf discolouration both increased as a function of time with both application rates of 2,4-D plus picloram and triclopyr. In each case the high application rate had a faster deleterious effect than the low application rate. By 20 days after herbicide application the trees treated with the high rates of the herbicides had no living leaves, although defoliation did not occur until later. The low application rates were somewhat less effective however, with approximately 75% of the foliage becoming discoloured and adopting a drooping posture by the 20th day.

Treatment of Carolina poplar trees with either 2,4-D plus picloram or triclopyr had a more rapid deleterious effect on the appearance of the foliage, compared to white ash, even though the low and high application rates were proportionally lower than those used with the white ash trees (Table 4). With the exception of the low application rate of triclopyr, the remaining three treatments had a maximal effect on foliage appearance by 15 days after herbicide application.

From the determination of the herbicide contents of foliage discussed previously (Table 3) it is apparent that while the appearance of the white ash foliage did not change as quickly as that of Carolina poplar, in response to herbicide treatment, the foliage of the former contained higher concentrations of the applied herbicides, even as early as 3 days after herbicide application.

The effects of soil-application of 2,4-D plus picloram or triclopyr to white ash and Carolina poplar trees described above are similar to those reported previously after foliar-application of these herbicides (OHRD Report No 83-88-K). Although in this previous study the foliage of both white ash and Carolina poplar trees was sprayed (to leaf drip) with either

TABLE 4

OBSERVATIONS ON THE EFFECTS OF SOIL-APPLICATION OF THE HERBICIDES 2,4-D PLUS PICLORAM AND TRICLOPYR ON THE APPEARANCE OF WHITE ASH AND CAROLINA POPLAR TREES

| Species | Herbicide Treatment | 2,4-D Plus Picloram | | | | | | Triclopyr | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White Ash | Rate Herbicide Applied | Low(ALDP) | | | High(AHDP) | | | Low(ALT) | | | High(AHT) | | |
| | Days After Herbicide Applied | +10 | +15 | +20 | +10 | +15 | +20 | +10 | +15 | +20 | +10 | +15 | +20 |
| | Effect Observed: | | | | | | | | | | | | |
| | Leaf discolouration | 0 | 1 | 2 | 1 | 2 | 3 | 0 | 2 | 2 | 1 | 1 | 3 |
| | Loss of leaf turgor | 0 | 1 | 2 | 1 | 1 | 3 | 0 | 1 | 2 | 1 | 2 | 3 |
| Carolina Poplar | Rate Herbicide Applied | Low(PLDP) | | | High(PHDP) | | | Low(PLT) | | | High(PHT) | | |
| | Leaf discolouration | 2 | 3 | 3 | 3 | 3 | 3 | 1 | 2 | 2 | 2 | 3 | 3 |
| | Loss of leaf turgor | 2 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 2 | 3 | 3 |

N.B. The trees in each treatment group were scored collectively on a scale of 0 (no effect) to 3 (maximum effect) at the times indicated.

the 2,4-D plus picloram solution used in field applications by Ontario Hydro at present, or the triclopyr solution recommended by the manufacturers and used in field operations by US utilities. In this earlier study the foliage on the Carolina poplar trees also became discoloured and lost turgor more rapidly than did the foliage on white ash trees, when both were sprayed with either 2,4-D plus picloram or triclopyr.

Both 2,4-D and picloram have been reported to reduce the chlorophyll content of herbicide-sensitive plants (Sharma and Vanden Born, 1972; Bruinsma, 1961) and this would lead to discolouration. Various investigators have described the rapid development of a permanently wilted condition in plants after application of 2,4-D (see Bradbury and Ennis, 1952).

### 3.4 Effects Of Soil-Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr On The Stomatal Diffusion Resistance Of Leaves Of White Ash And Carolina Poplar Trees

The relationship between stomatal diffusion resistance and transpiration in photosynthetic tissue has been described previously (OHRD Report No 83-88-K). The stomatal resistance ($r_s$) may be taken as a measure of the transpiration rate (one is inversely proportional to the other). Thus a small $r_s$ value corresponds to a high rate of transpiration. Transpiration is the loss of water from plants in the form of vapour. The rate of transpiration provides some indication of the level of metabolic activity in the leaf, in that although basically controlled by physical factors, it is partly controlled by plant structure and stomatal behaviour (Kramer and Kozlowski, 1979). Direct or indirect effects of herbicides on stomatal metabolism would result in changes in the rate of transpiration (and thus the stomatal diffusion resistance). The effects of soil-application of the herbicides 2,4-D plus picloram and triclopyr on the stomatal diffusion resistance of leaves of Carolina poplar and white ash trees are shown in Table 5. The values for $r_s$ reported for the control Carolina poplar and white ash trees at 2 days after herbicide application are similar to the values obtained in a previous study (OHRD Report No 83-88-K). All the herbicide treatments with both Carolina poplar and white ash resulted in a significant increase in the $r_s$ values obtained at 2 days (and thus a decrease in the rate of transpiration). By seven days however the control $r_s$ values for both Carolina poplar and white ash had increased dramatically, and for both species the only treatments which had a significant effect on $r_s$ were the high 2,4-D plus picloram and high triclopyr treatments with Carolina poplar. The high control values were probably the result of increased ozone levels experienced after these experiments were initiated. Both white ash and Carolina poplar control trees showed characteristic ozone injury

TABLE 5

EFFECT OF THE SOIL-APPLIED HERBICIDES 2,4-D PLUS PICLORAM OR TRICLOPYR ON THE STOMATAL DIFFUSION RESISTANCE ($r_s$) OF LEAVES OF CAROLINA POPLAR AND WHITE ASH TREES

| Tree Species | Treatment* | Days after herbicide treatment 2 — $r_s$ (sec $cm^{-1}$) — 7 | |
|---|---|---|---|
| Carolina Poplar | PLDP | 25.0 ±6.0(c) | 169.3 ±33.3(c) |
| Carolina Poplar | PHDP | 18.8 ±7.1(bc) | 81.7 ±39.2(b) |
| Carolina Poplar | PLT | 12.7 ±9.5(b) | 33.5 ±14.8(a) |
| Carolina Poplar | PHT | 17.1 ±7.3(bc) | 106.7 ±52.1(b) |
| Carolina Poplar | Control | 5.1 ±4.7(a) | 27.6 ±15.0(a) |
| White Ash | ALDP | 20.3 ±20.2(b) | 248.0 ±98.9(a) |
| White Ash | AHDP | 17.4 ±4.7(b) | 160.8 ±49.1(a) |
| White Ash | ALT | 26.4 ±19.1(b) | 187.8 ±91.5(a) |
| White Ash | AHT | 19.1 ±6.7(b) | 157.5 ±41.3(a) |
| White Ash | Control | 5.2 ±6.9(a) | 124.2 ±40.4(a) |

* Abbreviations used for treatments are described in Table 1.

+ The values given represent the mean ± one standard deviation (n = 6). For each of the two tree species values within a column followed by a different letter are significantly different at the 5% level using a multiple range test.

symptoms, with small areas of chlorotic tissue appearing on the upper leaf surfaces (Jacobson and Clyde Hill, 1970). There is evidence that ozone reduces $CO_2$ uptake and water vapour loss by inducing stomatal closure (Tingey and Taylor, 1982). The ozone injury symptoms were not visible on leaves produced on the trees later in the experiment. Foliar application of 2,4-D plus picloram or triclopyr to white ash and Carolina poplar trees has been reported to cause an increase in $r_s$ values in previous studies (OHRD Report No 83-88-K). The rapid effects of soil-application of the herbicides on transpiration (an increase in $r_s$ values) observed in the present study were probably caused by the presence of herbicide in the leaves, as described in Table 3. Within 3 days of herbicide application, herbicide had been translocated into the leaves and was inhibiting stomatal metabolism.

### 3.5 Effects Of Soil-Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr On The Respiration Of Root Segments From White Ash And Carolina Poplar Trees

Although none of the herbicide treatments had a significant effect on the respiration of root segments from Carolina Poplar by three days after herbicide application, by ten days all treatments caused a significant reduction in the respiration rate (Table 6). In white ash however, with the exception of the high application rate of triclopyr which caused a reduction in respiration after three days but not ten days, none of the herbicide treatments had a significant effect on respiration at three or ten days. These results would suggest that white ash roots are far more tolerant of all the herbicides than Carolina poplar roots, since the concentrations of the herbicides in white ash were higher than in Carolina poplar (Tables 1 and 2). Mitchell and Stephenson (1973) have reported a differential sensitivity of the roots to picloram. Inhibition of respiration in root segments of Carolina poplar and white ash to which 2,4-D plus picloram or triclopyr had been applied to the foliage has been observed previously (OHRD Report No 83-88-K) and of the two species Carolina poplar appeared the more susceptible.

### 3.6 Survival Of White Ash And Carolina Poplar Trees In The Field After Soil Application Of The Herbicides 2,4-D Plus Picloram And Triclopyr

Fifteen trees from each treatment group of white ash and Carolina poplar were transferred to the field in August 1983 and their survival monitored in the spring of 1984. Of the fifteen white ash control trees fourteen had survived and were in leaf when observations were made. None of the trees from the four herbicide treatments were alive. Of the fifteen Carolina poplar control trees, thirteen had survived while none of the trees

TABLE 6

EFFECT OF THE SOIL-APPLIED HERBICIDES 2,4-D PLUS PICLORAM OR TRICLOPYR ON THE RESPIRATION OF ROOT SEGMENTS OF CAROLINA POPLAR AND WHITE ASH TREES

| Tree Species | Treatment* | Days after herbicide treatment<br>3<br>- n moles $O_2$ consumed $min^{-1}g^{-1}$ fresh weight+- | <br>10 |
|---|---|---|---|
| Carolina Poplar | PLDP | 172 ± 52(a) | 128 ± 72(b) |
| Carolina Poplar | PHDP | 61 ± 3(a) | 99 ± 50(b) |
| Carolina Poplar | PLT | 144 ± 45(a) | 87 ± 15(b) |
| Carolina Poplar | PHT | 121 ± 81(a) | 99 ± 36(b) |
| Carolina Poplar | Control | 158 ± 45(a) | 239 ± 66(a) |
| White Ash | ALDP | 175 ± 13(a) | 260 ± 16(a) |
| White Ash | AHDP | 149 ± 41(a) | 149 ± 89(a) |
| White Ash | ALT | 141 ± 7(a) | 154 ± 92(a) |
| White Ash | AHT | 94 ± 23(b) | 136 ± 75(a) |
| White Ash | Control | 175 ± 33(a) | 157 ± 36(a) |

* Letters describe treatment in abbreviated form (see Table 1).

\+ The values given represent the mean ± one standard deviation (n = 3). For each of the two tree species values within a column followed by a different letter are significantly different at the 5% level using a multiple range test.

from the four treatment groups were alive approximately 10 months after herbicide treatment. These observations indicate that for both tree species while the respective low application rates of either 2,4-D plus picloram or triclopyr were effective in killing the experimental trees, the high application rates, which were ten-fold greater than the low rates in each case, provided far more herbicide than was required to cause death in the long term. The amount of herbicide available for root pick-up after application to the soil in which the plant is growing will vary as a function of the adsorption-desorption characteristics of the herbicide for the experimental soil being used (Hance, 1980). Although the low application rates were found to be totally effective, the minimum application rates of the herbicides necessary to cause tree death remain unknown.

The high rate of survival of the control white ash and Carolina poplar trees also indicated that the stress resulting from the transplantation procedure, from greenhouse to field, was minimal.

## 4.0 CONCLUSIONS

1. Application of the herbicides 2,4-D plus picloram or triclopyr to the soil in which white ash and Carolina poplar trees were growing resulted in an inhibition of transpiration in both species within two days, suggesting a rapid uptake and translocation of herbicide to the leaves. The concentrations of the various herbicides within the leaves after three days were low, particularly for Carolina poplar, suggesting a high sensitivity of transpiration to inhibition.

2. White ash roots appeared more tolerant than Carolina poplar to the herbicides applied, in that root respiration was less sensitive to inhibition by the herbicides. The concentrations of the three herbicides in the roots of white ash were higher than those in Carolina poplar, and reflect the larger amounts of herbicides applied to the soil in the first instance.

3. There appear to be differences in the manner in which the different herbicides are taken up by the roots of the two species. Picloram and perhaps triclopyr uptake seems to be time-dependent, certainly from 3 to 10 days after application, while 2,4-D uptake appears to be time-independent over the same period.

4. The herbicides taken up by both species were effective in causing death of the trees over the long term. The minimum concentrations of herbicides necessary to cause root and ultimately tree death remain to be determined.

5. The concentrations of picloram and triclopyr found in the roots of white ash and Carolina poplar respectively after soil-application are somewhat lower than those reported previously after foliar-application. This suggests that in general foliar-application may indeed be as efficient as soil-application as a method of getting herbicide to the primary target in woody species, the root system. However there remain differences in the efficiency of uptake and translocation of picloram, triclopyr and 2,4-D within and between species.

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